EXTRACTION OF ANTIOXIDANT FROM KUNDUR PLANT

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ABSTRACT

Kundur or its scientific name *Benincasa Hispida*, is a member of cucurbitaceae family and it is categorized as one of the more famous crops that is grown primarily for its fruits. These plants are usually recognized for their nutritional and medicinal properties. Commonly, the antioxidant from plant has been extracted by conventional extraction such as Soxhlet extraction and includes supercritical carbon dioxide extraction. However, those methods have a lot of disadvantages such as the process need high temperature and low pH which the result may come in acidic condition and the process is difficult to achieve high percentage of yield in the product. Therefore, another type of extraction must be conducted to replace the entire problem mentioned before. So, enzymatic-assisted extraction has been choosing to replace all the conventional extraction method. Mostly, enzymatic extraction gives a lot of advantage compared to conventional extraction such as the process will give more yields in the product.The present study is to investigate the effectiveness of two methods either enzymatic extraction or Soxhlet extraction in obtaining of antioxidants from the Kundur plant. Generally, there are three major parts in completing the researches on extraction of antioxidant from Kundur plant. The first part is the sample preparation. The sample consists of Kundur peels and leaves were grounded into fine powder in order to be used in enzymatic extraction. The second part is the extraction process by using conventional extraction. The conventional extraction that used in our researches is Soxhlet extraction. Firstly, the samples were prepared in the Soxhlet apparatus. The oil were obtained by removal the excess of solvent by using the rotary evaporator. The reading of absorbance was obtained by usingUV–vis spectrophotometer. The third part is the enzymatic extraction. For this part, the phenolic compound has been extracted in order to get the absorbance of the antioxidant inside of the sample. The sample was prepared in different temperature which is in the range from 20◦C to 60◦C and different in time which is from 3 until 10 hours in order to obtain the optimum parameter for the extraction process. The phenolic content of the samples was determined by using UV–vis spectrophotometer. The fourth part is the antioxidant determination. The fruit extract was reacted with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the absorbance was taken about 515 nm. The results shows that the kundur peels have antioxidant activity inside the sample. The reading of the recorded value of the absorbance shows that the linear range was lower than the standard curve. The standard curve was linear at 25 and 800 µM. These results suggest that it is possible to produce antioxidant from Kundur content, peel and seedand it can expands the usage of Kundur plant and it also can reduce the cost in production of antioxidant in Malaysia.

ABSTRAK

Kundur ataupun nama saintifiknya *Benincasa Hispida* adalah merupakan salah satu keluarga *cucurbitaceae* dan ianya telah dikategorikan sebagai salah satu tumbuhan yang terkenal di mana ditanam untuk mendapatkan buahnya. Tumbuhan ini dikenali hanya untuk kandungan nutrisi dan tujuan perubatan. Kebiasaanya, antioksida daripada tumbuhan ini telah diekstrakkan dengan menggunakan pengekstrakkan secara konvensional contohnya Soklet pengekstrakkan dan termasuk karbon dioksida pengekstrakkan. Walaubagaimanapun, cara tersebut mempunyai banyak kekurangan contohnya proses tersebut memerlukan suhu yang tinggi dan pH yang rendah dimana akan menyebabkan hasil mungkin berada dalam keadaan asid serta sukar untuk mendapatkan peratusan hasil yang tinggi di dalam produk. Oleh itu, kaedah pengekstrakkan yang lain hendaklah dilakukan untuk mengantikan masalah tersebut. Justeru, pengekstrakkan berasaskan enzim telah dipilih untuk menggantikan kaedah ekstrak secara konvensional. Pengekstrakkan berasaskan enzim memberikan lebih banyak kelebihan berbanding kaedah pengekstrakkan secara konvensional seperti produk yang terhasil lebih banyak. Objektif kajian dijalankan adalah untuk menyiasat keberkesanan dua kaedah tersebut sama ada pengekstrakkan berasaskan enzim atau pengekstrakkan secara konvensional dalam mendapatkan antioksida daripada pohon Kundur. Secara umumnya, terdapat tiga bahagian dalam melengkapi kajian ini berdasarkan pengekstrakkan antioksida daripada pohon Kundur. Bahagian pertama ialah penyediaan sampel. Sampel ini mengandungi kulit dan daun Kundur yang telah dihancurkan sehingga menjadi serbuk dan akan digunakan untuk proses pengekstrakan enzim. Bahagian seterusnya adalah proses pengekstrakan dengan menggunakan proses pengestrakan konvensional. Ekstrak konvensional yang digunakan dalam penyelidikan ini ialah pengekstrakan Soxhlet. Dalam proses pengekstrakan Soxhlet, perkara pertama yang akan dilakukan adalah penyediaan sampel menggunakan alatan Soxhlet. Hasil pengestrakan Soxhlet adalah didalam bentuk minyak yang dihasilkan melalui dengan mengeluarkan lebihan bahan pelarut menggunakan penyejat putar. Seterusnya, bacaan "absorbance" akan didapatkan menggunakan sistem UV-vis spektrofotometer.Bagi bahagian pengekstrakan enzim pula, sebatian phenolic telah diekstrak demi mendapatkan bacaan "absorbance" bagi kadar antioksida yang terdapat di dalam sampel tersebut. Sampel-sampel tersebut telah disediakan dengan kadar waktu yang berlainan iaitu didalam tempoh 3 hingga 10 jam dan juga kadar suhu yang berlainan, dari 20◦C hingga ke 60◦C untuk mendapatkan parameter yang optimum bagi proses pengekstrakan ini. Kadar kandungan phenolic yang tedapat didalam sampel tersebut telah diukur menggunakan "UV-Vis Spectrophotometer". Seterusnya pula, bagi bahagian penentuan kadar antioksida, ekstrak kundur tersebut telah ditindakbalaskan dengan "2,2-diphenyl-1-picrylhydrazyl (DPPH)". Kemudian, bacaan "absorbance: telah diambil pada 515nm dan keputusan tersebut menunjukkan bahawa terdapatnya kesan antioksida dalam ekstrak kundur tersebut. Nilai bacaan yang telah direkodkan menunjukkan bahawa nilai "absorbance" yang diperoleh dalam "linear range" adalah lebih rendah daripada apa yang direkodkan dalam "standard curve". Dalam "standard curve" bagi eksperimen ini, nilai linear adalah dari 25 hingga ke 800 µM. Hasil eksperimen in membuktikan bahawa ia adalah amat mungkin untuk menghasilkan antioksida dari daun dan kulit Kundur. Akhir sekali, dengan mengikut cara dan proses yang betul, penggunaan Kundur untuk menghasilkan antioksida boleh diperluaskan dan kos penghasilan antioksida dalam Malaysia juga boleh dikurangkan.

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1 INTRODUCTION

1.1 Background of Study

Curcurbitacea is one of the most genetically diverse groups of food plants whereas the families of this group family are frost sensitive and drought-tolerant. The example of *Cucurbit* family include; gourd, melon, cucumber, squash and pumpkin. The fruits from this group species are valued nutritional and medical purpose). *Benincasa hispida* is categorizing one of the *cucurbitacea* families. *Benincasa hispida* fruit is a good source of a lot of valuable nutrients such as organic acid, natural sugar, vitamins, amino acids and mineral elements. The fruit has been widely used for the treatment of ulcer, epilepsy, diabetic complications, hypertension, nervous disorders and Alzheimer disease in the traditional medicine system of Asian communities (Farooq *et al*., 2011). Several analysis and investigation focused on the biologically active components of benincasa species and it's proved that antioxidant activity on different tissues like liver and brain have been seen. (Madanna *et al.,* 2013). Currently, the uses of this plant are mostly due to its food and medicinal benefits. These types of plant contain a lot of antioxidant especially inside of its seed and peel. Many done investigation have be extract all the antioxidant from the Kundur plant in order to cure disease.

1.2 Motivation and Problem Statement

Free radical is a molecule that has an odd number of electrons. These free radicals are mostly caused by stress, illness, drugs and pollution. These free radicals can induce damage to cell, DNA, lipids and protein. It also play key role in cancer, cardiovascular disease and neurological disorders. To protect human body, and antioxidants are needed in order to against damages of free radicals due to their redox properties. (Madanna *et al*.,2013).Antioxidants are the compound that can delay or inhibit the oxidation of molecule and capable of stabilizing, or deactivating the free radicals before it can attack the cells. Phenolic compound is one of the stable antioxidant and it can be found in plants and fruits. The presences of flavonoid inside of the phenolic compound will

make the phenolic compound act as good cell cycle inhibitor. This makes flavonoids have beneficial implications in human health due to their antioxidant activities and free radical scavenging abilities. (Smith,2006).

Nowadays, there has been an interest by the industry and a desire by consumers to replace synthetic compounds with natural antioxidant alternatives. It can reduce the cost of industry, making antioxidant. We can use the natural alternatives sources of antioxidant for our health. We also can identify the sources by using laboratory equipment such as HPLC and also GC. Lastly, the extraction of ascorbic acid from Kundur can expands the usage of Kundur plant and it can reduce the cost in production of antioxidant in Malaysia.

Curcurbitacea is one of the most genetically diverse groups of food plants whereas the families of this group family are frost sensitive and drought-tolerant. The example of Cucurbit family include; gourd, melon, cucumber, squash and pumpkin. The fruits from this group species are valued nutritional and medical purpose). *Benincasa hispida* is categorizing one of the *cucurbitacea* families. *Benincasa hispida* fruit is a good source of a lot of valuable nutrients such as organic acid, natural sugar, vitamins, amino acids and mineral elements. The fruit has been widely used for the treatment of ulcer, epilepsy, diabetic complications, hypertension, nervous disorders and Alzheimer disease in the traditional medicine system of Asian communities (Farooq *et al*.,2011). Several analysis and investigation focused on the biologically active components of *benincasa* species and it's proved that antioxidant activity on different tissues like liver and brain have been seen. (Madanna *et al*.,2013). Currently, the uses of this plant are mostly due to its food and medicinal benefits. These types of plant contain a lot of antioxidant especially inside of its seed and peel. Many done investigation have be extract all the antioxidant from the Kundur plant in order to cure disease.

Numerous studies and researches have been done in order to find the total antioxidant activity and total phenolic content in Kundur plant such as Chopra et al.(1956) , Grover and Rathi,(1994), Grubben (2004), Yadav *et al*.(2005) and Zaini *et al*.(2011). All the previous studies basically used conventional extraction as their main extraction process. Although all the extraction process is understood, however there is no research has be performed by applying enzymatic extraction on Kundur plant. Therzadeh & Karimi (2007) stated that The advantages of enzymatic extraction are

can be undergo in mild condition of temperature and pH, high yield of hydrolysis and low toxicity of the hydrolyzates formed. In other study, it have stated that the conventional extraction are the process need to be carried out in high temperature and in lower pH. This will make the outcome product will be more in acidic and corrosive condition. Besides, during the process of conventional extraction, several inhibitor compound can be formed and the time for conventional extraction sometimes need to be carry out in long period of time. Therefore, the aim of this study is to apply the enzymatic extraction as the main extraction process rather than conventional extraction to Kundur plant. This paper aims for several objectives which are (1) to apply enzymatic extraction and determine the optimum parameter in enzymatic extraction (2) to analyze the antioxidant scavenging activity and total phenolic content from the Kundur plant (3) to compare the enzymatic extraction with conventional extraction.

1.3 Objective of the Research

1) To identify the antioxidants from the Kundur plant
2) To apply enzymatic extraction and determine the optimum parameter in enzymatic extraction
3) To compare the enzymatic extraction with conventional extraction.

1.4 Scope of the Research

1) Study the Soxhlet extraction and enzyme-assisted aqueous extraction process
2) Determination of phenolic content
3) Determination of Antioxidant activity by DPPH

1.5 Rational of Significant Study

1) To expands the usage of Kundur plant
2) Save the amount of solvent used
3) Reducing the effect to the environment
4) Minimize the extraction time

1.6 Organization of This Thesis

Chapter 2 provides a description of the characteristic and the content of *Benincasa Hispida*. A general description related to the uses and applications of this plant in industry are presented. This chapter also provides a brief discussion of the conventional extraction method and enzymatic hydrolysis extraction method. A summary of the previous experimental work on the optimum parameter of the extraction process which is time and temperature also provided.

Chapter 3 gives a review about the sample preparation of Kundur, Soxhlet and enzymatic extraction process and determination of antioxidant by using two different assay. The sample preparation of Kundur is prepared in different time and temperature to obtain the optimum parameter. Both of conventional and enzymatic extraction have been done to obtain the result of antioxidant in different type of extraction. The antioxidant activity is determined by using DPPH assay.

Chapter 4 shows the review of the result of antioxidant activity inside of the Kundur sample. A preliminary result about the standard curve of the ascorbic acid and gallic acid also provided. The result about the optimum parameter condition of enzymatic extraction, total phenolic content, activity of antioxidant activity and the comparison of enzymatic extraction also have been provided.

Chapter 5 shows the review of the overall conclusion of this study and the recommendation that can be taken in order to improve this study in the future.

2 LITERATURE REVIEW

2.1 Overview

This paper presents the experimental studies extraction of antioxidant from Kundur plant. In order to identify the antioxidant inside the Kundur plant, both of extraction which are conventional and enzymatic hydrolysis extraction process will be done. Basically, conventional extraction such as Soxhlet extraction use chemical solvent such as ethanol as their extraction medium while the enzymatic hydrolysis extraction use enzyme such as cellulase as their medium of extraction. Various temperature and time have been done in order to get the optimum parameter of the extraction. The optimum parameter for the extraction process time is 80 minutes according to Thoo et al, (2010) while the optimum temperature for the process is basically run in the lower temperature (Chan et al, 2009). The phenolic content inside of the Kundur was identified by using Folin reagent and the phenolic compound is 74. 83 ± 1.42 mg GAE/g extract weight (Noriham,2012). The total antioxidant that have been extracted is mostly abundant in enzymatic extraction rather than conventional extraction after the sample was tested with 2,2-diphenyl-2-picrylhdrazyl radical (DPPH assay).

2.2 Kundur- Introduction

Benincasa hispida is one of the species of cucurbitaceae family. According to Whitaker & Bohn, 1950, Cucurbitaceae (cucurbit) family is one of the most genetically diverse groups of food plants in the plant kingdom. The plants belonging to this family are frost-sensitive, drought-tolerant, and intolerant to wet and poorly drained soils. Some prominent cucurbit family members are gourd, melon, cucumber, squash and pumpkin (Robinson & Decker-Walters, 1999). This type of plant is grown primarily basically for its fruits. Table 2.1 shows the classification of Kundur plant according to the data from the United States Department of Agriculture (USDA, 2009). Kundur fruit is known as an important vegetable in India, China, Philippines and elsewhere in Asia.

Table 2.1 Classification of Kundur (Benincasa hispida).

Kingdom Plantae	Plants
Subkingdom	Tracheobionta — Vascular plants
Superdivision	Spermatophyta — Seed plants
Division	Magnoliophyta — Flowering plants
Class	Magnoliopsida — Dicotyledons
Subclass	Dilleniidae
Order	Violales
Family	Cucurbitaceae — Cucumber family
Genus	Benincas Savi — benincasa
Species	Benincasa hispida

This plant also has a great potential for food production. For commercial purposes in Malaysia, just two cultivar (round shape and elongated) of wax gourd are grown (Mohd Zaini, N.A et al, 2010).

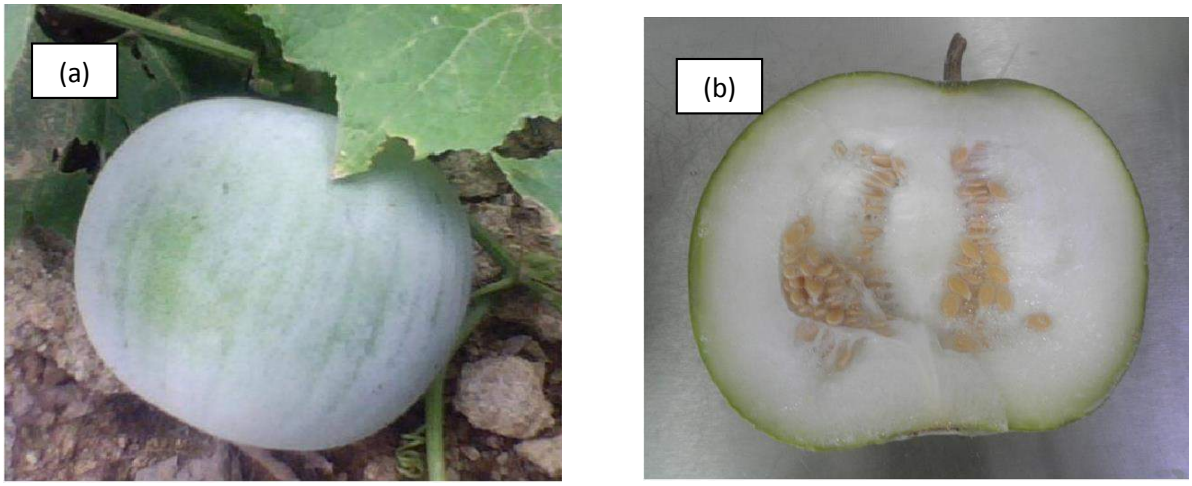

Figure 2.1 Whole (a) and half-cut (b) Kundur (Benincasa hispida) fruit

This plant is also known as Kundur (Malay), ash gourd or winter melon (English), Bhuru Kolu or Safed Kolu (Gujarati), Petha (Hindi), Kushmanda (Sanskrit), Dōngguā (Chinese) and Beligo (Indonesian). (Sew,C.C et al,2010). Mature winter melon can be identified when it's thickly deposited hairs with easily removable waxy bloom while the young fruit has succulent, fleshy and hairy attributes. The shape of the fruit can be cylindrical, globular or oblong and the flesh of the mature fruit is white, juicy and spongy. (Mohd Zaini, N.A et al,2011).

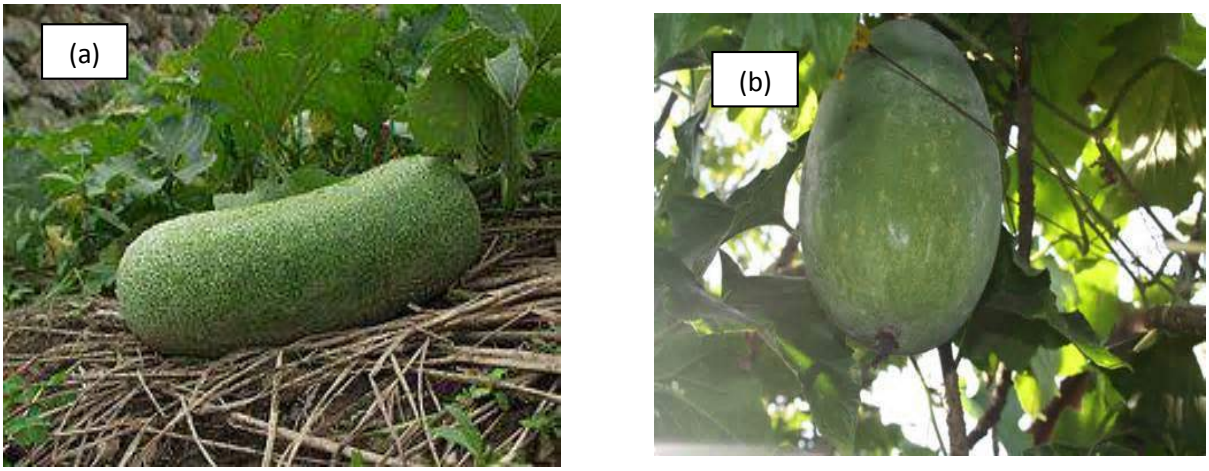

Figure 2.2 (a) Cylindrical Kundur fruit **(b)** globular Kundur fruit

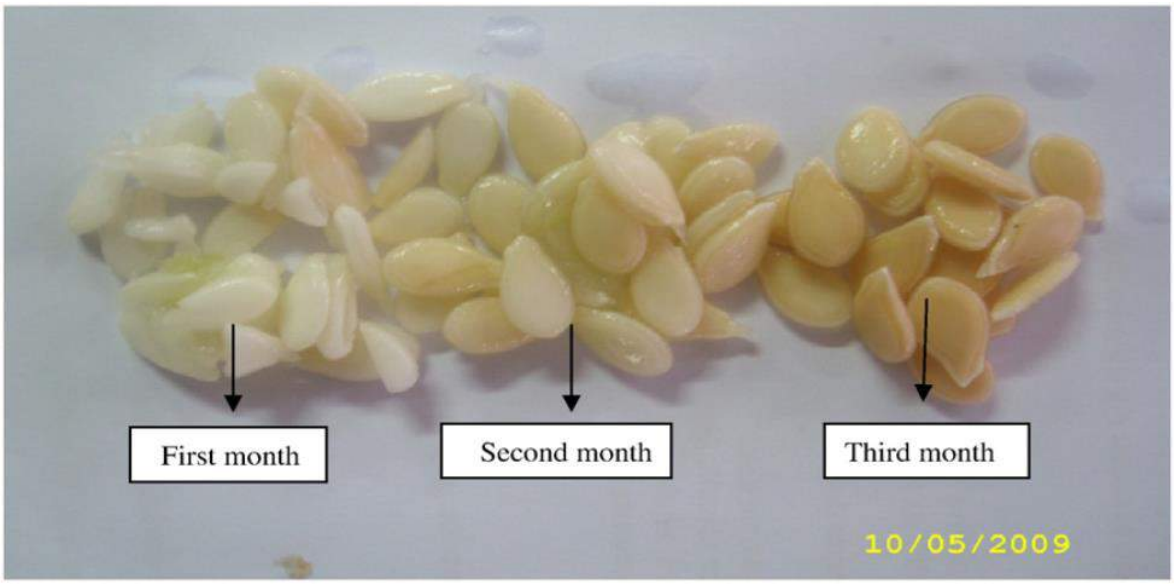

Figure 2.3 Color changes in Kundur (*Benincasa hispida*) fruit seed with maturity.

Bates & Robinson (1995) state that there are four recognized cultivars of winter melon fruit which are unrigged winter melon, ridged winter melon, fuzzy gourd and wax gourd. These types of winter melon are mainly characterized based on their size, shape, fuzziness, waxiness, and presence or absence of a dusty or ashy layer. In Malaysia, winter melon, with local name "Kundur" is mainly represented by two cultivars namely round and oval. However a hybrid-type round which the fruit is developed through breeding of the green winter melon genotype and fuzzy white gourd genotype is also have been grown widely in Malaysia (Zaini et al., 2011). Kundur fruit in Malaysia, although cultivated on a considerable area, is underutilized. Johor, Pahang, Perak Sabah, Kelantan and Selangor are some of the important states cultivating this fruit species mainly for vegetable purposes.

Almost all parts of *Benincasa hispida* (B.hispida) which are leaves, flower, fruit and seed have been used, either as food or as medicine. The young shoots, leaves and flowers can be used as vegetable. The immature as well as mature, large size fruits are often cooked as vegetable, stuffed and steamed or chopped into small blocks candied with sugar. Marr et al.(2007) stated that In China, India, Nepal, Cuba and Southeast Asian regions, the mature fruits are used in preparation of soups, while these are also sliced and eaten as cooked alone or with meat and as well as incorporated in the preparations of other dishes.

Many researches have been done in order to identify the uses of this plant. There have been research prove that Kundur fruit is a good source of valuable nutrients including organic acids, natural sugars, amino acids, vitamins and mineral elements. A number of biological and medicinal properties such as anti-obesity, anti-inflammatory, anti-diarrhoeal , anti-pyretic, anti-compulsive, antioxidant , anti-ulcer and diuretic have been ascribed Kundur (Grover et al., 2001; Rachchh & Jain, 2008).As a potential source of wide array of functional bioactive and therapeutics, the component inside of the plant such as phenolics, triterpenes, glycosides and sterols, the fruit has been widely used for the disease treatment. The disease such ulcer, epilepsy, diabetic complications, hypertension, nervous disorders and Alzheimer disease are mainly disease that can be treated by antioxidant inside of the Kundur plant.

According to Huang et al. (2004), the antioxidant activity of Kundur fruit show that the seed has the higher capacity for inhibition of linoleic acid oxidation and scavenging 2,2 diphenyl-1-picrylhydrazyl (DPPH) radicals compared to the peel, pulp and core of the fruit. This is because

the total phenolic contents and superoxide dismutase (SOD) activity of the seeds is higher. The fruit also exhibits anti-carcinogenic effects in vivo. Kundur fruit also give positive potential antioxidant activity on the kidney (Bhalodia et al,2011). Kundur fruit can reduce renal damage after ischemia or reperfusion injury of the kidney. Ischemia or reperfusion of the kidney is the major cause of acute renal failure and may be involved in chronic renal problems. This finding also supported by Mingyu et al (1995) which show that Kundur fruit has significant protection and blocking effects upon the kidney injury caused by mercury chloride. This might be the presence of polyphenolics such as flavones (iso-vitexin) inside of the Kundur fruit.

One of antioxidant that can be found in Kundur plant is flavonoid C-glycoside. Flavonoids also known as Vitamin P and citrin. It also classified in class of plant secondary metabolites (William,2004). According to the IUPAC nomenclature, they can be classified into three which are flavonoids, isoflavonoids and neoflavonoids. Flavonoids are most commonly known for their antioxidant activity in vitro. Industrial manufacturing such as food and consumers manufacturers have become interested in flavonoids for their possible medicinal properties, especially their putative role in prevention of cardiovascular diseases and cancers (Cushnie, 2011). Although physiological evidence is not yet established, the beneficial effects of fruits, vegetables, and tea or even red wine have sometimes been attributed to flavonoid compounds rather than to known micronutrients, such as vitamins and dietary minerals. Flavonoids might induce mechanisms that affect cancer cells and inhibit tumor invasion. In preliminary studies, cancer researchers proposed that smokers who ate foods containing certain flavonoids, such as the flavan-3-ols (catechins) found in strawberries and green and black teas, kaempferol from brussel sprouts and apples, and quercetin from beans, onions and apples, may have reduced risk of developing lung cancer (Serafini,2003).

2.3 Extraction Method

Many extraction methods can be done to extract one component from another component. Extraction is a major step for the isolation, identification and use of valuable compounds from different plants. The examples for extraction method are Soxhlet extraction, Supercritical fluid extraction, ultrasound extraction and enzymatic-assisted extraction. The choice of extraction technique is basically decided upon based on initial cost, operating cost, simplicity of operation,

amount of organic solvent required and sample throughout (Saim,N. et al, 1998). The range of approaches currently available makes the selection of the most appropriate extraction technique difficult. Soxhlet extraction is one of solid-liquid extraction process. Originally, Soxhlet extraction is applying leaching technique in the extraction process and it is one of the oldest ways of solid sample pretreatment. Basically in conventional Soxhlet, this extraction method used for the determination the value of component inside of the sample. The sample is placed in a thimble-holder. During operation, the thimble-holder gradually filled with condensate fresh solvent from a distillation flask (see Figure 2.4). When the liquid reaches the overflow level, a siphon aspirates the solute of the thimble-holder and unloads it back into the distillation flask, carrying the extracted analytes into the bulk liquid. This operation is repeated until complete extraction is achieved. This performance makes Soxhlet a hybrid continuous and discontinuous technique. In as much as the solvent acts stepwise, the assembly can be considered as a batch system; however, since the solvent is recirculated through the sample, the system also bears a continuous character (Luque,1994).

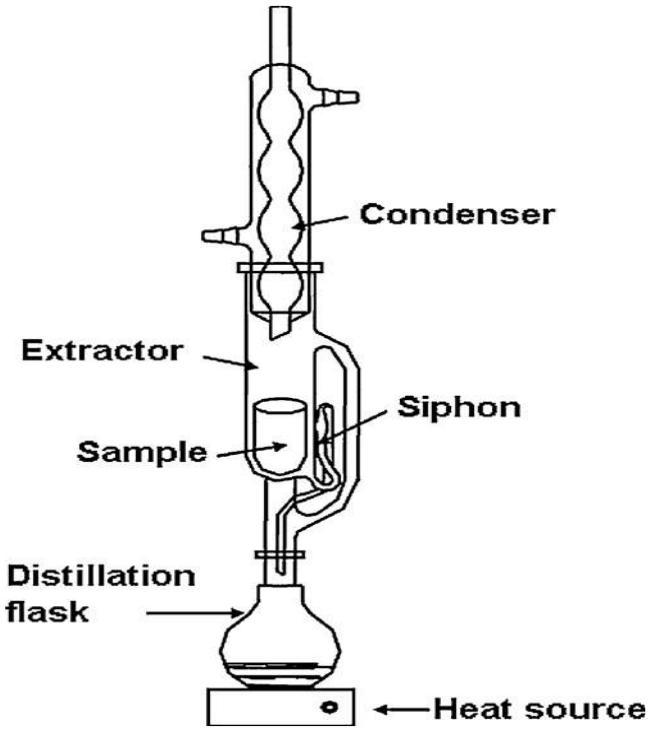

Figure 2.4 Conventional Soxhlet extractor.

According to Luque de Castro & Garcia-Ayuso (1998), Soxhlet extraction is one of the leaching techniques mostly used for a long time. According to Priego (2007), ultrasound assisted extraction is can improve the Soxhlet extraction process. Ultrasound-assisted methods are usually developed in a batch mode, discontinuous, and the shortening of the extraction time which is respect to that in the absence of ultrasounds, is due to an increase of both pressure and temperature (which improves solubility and diffusivity), both increasing the transport phenomena and displacing the partitioning equilibrium. The use of ultrasound-assisted extraction is advisable for thermolabile analytes which are altered under Soxhlet working conditions. In a number of comparisons, the efficiency of both the alternatives is similar but Soxhlet extraction provides better reproducibility .In other comparisons the efficiency of Soxhlet is higher than that of ultrasound-assisted extraction (Miller, 1988).

Enzyme-assisted extraction is a extraction method apply the study of metabolites releasing from biogenic materials. This type of extraction use enzyme to break down the linked-bond inside of the plant cell wall. Enzyme-assisted extraction has advantages of high efficiency, environmental friendship and easy operation process. It also been represented as an alternative way for natural product extraction (Fu et al., 2009). All the mechanism for enzyme-assisted extraction of phenolic compounds from residual sources is mostly based on the cell-wall degrading enzymes that can weaken or break down the cell wall. This will make the intracellular materials will more exposed for extraction. Enzyme-assisted extraction of natural functional compounds from plants is widely investigated in recent years for its advantages in high efficiency, easy operation, and environment friendship (Barzana,2002). Most of the works in this field utilize pectinase and cellulase to hydrolyze and degrade plant cell wall constituents in order to improve the release of intracellular contents. Another important factor in the extraction are the intrinsic property especially the solubility of the target compound, has seldom been concerned according to our knowledge. Low solubility of target compounds in the extractant leads to low extraction yield and require large amount of solvents, which largely impedes the economic efficiency in industry.

Supercritical fluid extraction (SFE) is the extraction process that involve of separating one component from another by using supercritical fluids as the extracting solvent. Besides, it also can be defined as fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure (Bimakr, 2012). Supercritical fluid extraction (SFE) has received considerable

attention as a promising alternative to conventional technology for separation of different valuable compounds from natural sources. According to Mitra et al, (2009), carbon dioxide (CO2) used as main supercritical fluid, sometimes modified by solvent such as methanol or ethanol. This gases also behaves as a gas in air at standard temperature and pressure (STP), or as a solid called dry ice when frozen. If the temperature and pressure are both increased from STP to be at or above the critical point for carbon dioxide, it can adopt properties midway between a gas and a liquid. More specifically, it behaves as a supercritical fluid above its critical temperature (304.25 K) and critical pressure (72.9 atm or 7.39 MPa), expanding to fill its container like a gas but with a density like that of a liquid. The suitable condition for supercritical CO2 extraction is above the critical temperature of 31°C and critical pressure of 74 bars (R.B. Johnson, 2003).Supercritical carbon dioxide (SC-CO2) has been the most commonly used solvent in the food and pharmaceutical industries, since it is non-toxic, non-flammable, chemically stable, inexpensive, environmentally acceptable and easily separated from the extract.

Ultrasound is acoustic (sound) energy in the form of waves having a frequency above the human hearing range. The highest frequency that the human ear can detect is approximately 20 thousand cycles per second (20 kHz). This is where the sonic range ends, and where the ultrasonic range begins. Ultrasound is used in electronic, navigational, industrial, and security applications. It is also used in medicine to view internal organs of the (Garcia & J.L., 2003).The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave. Cavitation bubbles are produced and compressed during the application of ultrasound. The increase in the pressure and temperature caused by the compression leads to the collapse of the bubble. With the collapse of bubble, a resultant ‘‘shock wave’ ’ passes through the solvent enhancing the mixing. Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the sample conmatrix, increasing the contact surface area between solid and liquid phase. This coupled with the enhanced mass transfer and significant cell disruption of cells via cavitation bubble collapse, increases the release of intracellular components into the bulk medium. The use of higher temperatures in UAE can increase the efficiency of the extraction process due to the increase in the number of cavitation bubbles formed (M.A., Palma, & Barroso, 2003). The application of ultrasound waves have also been reported (Suslick, 1998)to result in the formation of free radicals as a result of very short term local temperature and pressure increases, which may partially consume the antioxidants released

from the matrix. Therefore, the optimum duration of ultrasonication may be anywhere between very short and very long times. UAE of various analyses from a variety of organic and inorganic samples using different types of solvents have been reported in the literature. Ultrasonic bath and ultrasonic probe systems are the two most common devices used in ultrasound-assisted extraction. The UAE is carried out in three ways; indirect or direct sonication using an ultrasonic bath and direct sonication using an ultrasonic probe (Vinatoru, et al., 1997). Direct sonication is more effective on extracting solvent and solid. During indirect sonication, effects of ultrasound waves may be buffered due to the presence of a layer around material. Longer sonication times can be needed to get same extraction efficiencies as those obtained by indirect sonication. Wu, Lin, & Chau (2001) compared direct and indirect ultrasonication with Soxhlet extraction of ginseng saponins from ginseng roots and cultured ginseng cells. It was found that UAE was about three times faster than the traditional extraction method and direct sonication by the probe could provide much higher ultrasound energy to the samples than indirect sonication by the cleaning bath.

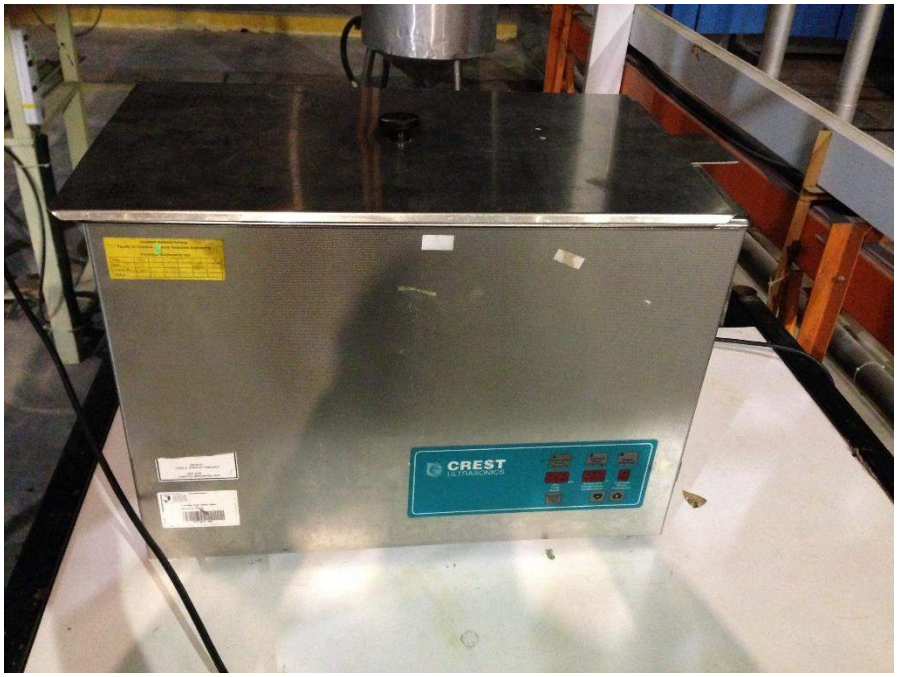

Figure 2.5 Ultrasonic Assisted Extractor

2.4 Enzyme-Assisted Extraction Advantages

Enzyme-based extraction of bioactive compounds from plants is a potential alternative to conventional solvent based extraction methods. Basically, solvent extraction and conventional extraction has more disadvantages rather than enzymatic-assisted extraction. Enzymatic hydrolysis can be carried out under mild conditions, whereas acid hydrolysis requires high temperature and low pH, which results in corrosive conditions. Besides, acid hydrolysis is difficult to achieve high yield of cellulose hydrolysis while enzymatic extraction can obtain almost 100% (Ogier et al. 1999). During enzymatic hydrolysis, the inhibitor compound did not formed while for the acid hydrolysis extraction, several inhibitory compounds are formed during the extraction process.

Enzymes are ideal catalysts to assist in the extraction, modification or synthesis of complex bioactive compounds of natural origin. Enzyme-based extraction is based on the inherent ability of enzymes to catalyze reactions with exquisite specificity, regioselectivity and an ability to function under mild processing conditions in aqueous solutions (Gardosi:, 2009). According to Meyer (2010), this method also offers the possibility of greener chemistry as pressure mounts on the food industry and even pharmaceutical companies to identify cleaner routes for the extraction of new compounds. Besides, a studies that have been done by Pinelo (2009) stated that enzymes have the ability to degrade or disrupt cell walls and membranes, thus enabling better release and more efficient extraction of bioactives. Enzyme-assisted extraction methods are gaining more attention because of the need for eco-friendly extraction technologies. A quantitative characteristic of enzymatic processing in industry is represented in the literature by relatively few enzyme applications. These include laccase applied in bleaching in the pulp and paper industry , protease/ lipase applied in leather making , lipase applied in the production of skin care products (Veit, 2004), and phospholipase applied in degumming of soybean oil (DeMaria, 2007). A particularly useful application of enzymes increases the effect of solvent pre-treatment and either reduces the amount of solvent needed for extraction or increases the yield of extractable compounds. Enzymes such as pectinases, cellulases and hemicellulases are widely used in juice processing and beer clarification to degrade cell walls and improve juice extractability. The disruption of the cell wall matrix also releases components such as phenolic compounds into the juice, thus improving product quality. Moreover, Barzana (2002) revealed that enzyme-assisted extraction methods have been shown to achieve high extraction yields for compounds including polysaccharides, oils,

natural pigments, flavours and medicinal compounds. Recent studies on enzyme assisted extraction have shown faster extraction, higher recovery, reduced solvent usage and lower energy consumption when compared to non-enzymatic methods. In this review, we provide a brief description of quantitative screening of enzyme applications, comparing the overall energy consumption of systems involving enzymatic processing to systems involving conventional chemical processing.

For Soxhlet extraction, a large consumption needs to be done as well as a long sample treatment (Luque de Castro & Garcia- Ayuso, 1998). Large amount used will cause a lot of solvent wasted which not only expensive to dispose and also can cause additional environmental problems. Supercritical fluid extraction also has several disadvantages such as essential oils from plant undergo incomplete extraction, high operating temperatures with the consequent breakdown of thermally labile components, the hydration reactions of chemical constituents will be promoted, and need post extraction process to remove water (Chyau et al. (2007). Another disadvantages of this extraction process are the process itself required elevated pressure, compression of solvent requires elaborate recycling measures to reduce energy costs and high capital investment for equipment. While, for ultrasonic baths, although it is more widely used devices, they have two main drawbacks that considerably decrease experimental repeatability and reproducibility (Luque-Garcia and Luque de Castro, 2003):

- Lack of uniformity in distribution of ultrasound energy (only a small fraction of total liquid volume in the immediate vicinity of the ultrasound source experiences cavitation)
- Decline of power with time, so the energy supplied to bath is wasted.

2.5 Effect of Temperature and Concentration

The enzymatic extraction need to be run in suitable temperature. According to Chan et al. (2009), at higher extraction temperature, the loss in antioxidant capacities of plant extracts was due to degradation of phenolic compounds that mobilized at low temperature. So, if the phenolic compound undergoes extraction process at high temperature, the amount of antioxidant activity

will be lowered compared to those which were extracted under low temperature. The antioxidant capacity also depends on the structure and interaction between extracted phenolic compounds (Huang et al., 2005). Therefore, further study on identification of phenolic compound in Kundur plant which is extracted at different temperature with respect to their antioxidant mechanism should be carried out. Another parameter that affects the extraction yield is extraction time. The cost can be saved by not wasting time in order to gain the yield of extraction. A study was carried out by Silva et al., (2007) show that the increasing of excess extraction time will reduce the amount of yield of extraction compound. The optimum extraction time for antioxidant compounds is varies with antioxidant capacity. Thoo et al, (2010) show that the best extraction time in order to get antioxidant capacity is 80 minutes. From the study by Maisuthisakui and Pongsawatmanit, (2004), the yield of the extract and total phenolic content were almost constant after 3 hours of extraction time at room temperature. However, Chew K.K, (2011) gives another results which is the extraction was the best at 300 minutes. Therefore, in order to get the exact time for enzymatic-assisted extraction time for Kundur sample must be done.